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THE ULTRASTRUCTURE OF COELOMOCYTES OF THE SEA STAR *DERMASTERIAS IMBRICATA*

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It has been previously reported that the sea star, *Dermasterias imbricata*, possesses a true adaptative cell-mediated immune response, as reflected by the specific chronic rejection of integumentary allografts (Karp and Hildemann, 1976). The reaction appears to be mediated by infiltration by phagocytic cells. Since the predominant phagocyte in the sea star is the coelomic amoebocyte (Boolootian and Giese, 1958; Endean, 1966), this cell may mediate the graft reaction. This hypothesis is supported by the recent report of Bertheussen (1979) that phagocytic amoebocytes of the sea urchin *Strongylocentrotus drobachiensis* demonstrate *in vitro* cytotoxic reactions against both allogeneic and xenogeneic echinoid cells. Although several excellent studies characterize the coelomocytes of holothurians and echinoids (Hetzel, 1963; Johnson, 1969a, b, c; Chien *et al.*, 1970; Fontaine and Lambert, 1973, 1977; Bertheussen and Seljelid, 1978; Bertheussen, 1979), only sparse information deals with asteroids. To further understand the immunologic capacities of the sea star, *Dermasterias*, and to compare them with those of other animals, we have characterized the ultrastructure of the coeolomic amoebocyte and have reaffirmed its phagocytic role. Ultrastructural observations on Tiedemann's bodies were also made to determine whether or not the cells of this organ participate in phagocytic activities. We experimented on animals stressed by intermittent extraction of coelomic fluid to follow the recovery of coelomocytes, and to determine whether or not this recovery is due to cell division in the circulating coelomic fluid.

Materials and Methods

Animals

Specimens of *Dermasterias imbricata* collected off the California coast were purchased from Pacific Biomarine (Venice, CA) and maintained at 15°C in aquaria containing artificial sea water adjusted to a specific gravity of 1.03–1.04 (Instant Ocean, Aquarium Systems, Wickliffe, OH). The animals were fed lake smelt three times per week. Animals exhibited active locomotion and extension of tube feet, indicating good health.

Isolation of coelomocytes

Coelomic fluid (containing coelomocytes) was obtained by inserting a tuberculin syringe with a #27 gauge needle into the interradial area through the aboral surface of the central disc. Before fluid withdrawal, individual animals were weighed on an Ohaus Harvard trip balance after they were drained of excess sea water for 30 sec.

Coelomic fluid was rapidly added to equal volumes of a fixative solution containing 6% glutaraldehyde and 10 mM ethylene bis (oxyethylene-nitrilo) tetra-acetic acid (EGTA) in Ca^{2+}-free artificial sea water (M.B.L. formula; Cavanaugh, 1964) adjusted to pH 7.4. The addition of the Ca^{2+} chelator and use of Ca^{2+}-free sea water minimized aggregation of coelomocytes (Edds, 1977). Coelomocytes were viewed at ×40 magnification on a Zeiss light microscope and their numbers determined with a hemocytometer.

Light microscopy

Coelomic fluid was placed on glass slides and coelomocytes were observed by phase contrast microscopy as smears or hanging drop preparations. Living coelomocytes were stained for lysosomes with 0.1% neutral red. Cells were allowed to adhere to glass slides coated with bovine serum albumin, then dried and treated with Giemsa stain to aid observations on nuclear configurations.

Electron microscopy

Coelomocytes were prepared for scanning electron microscopy (SEM) and transmission electron microscopy (TEM) by fixation in 3% glutaraldehyde in M.B.L.-formula sea water, post-fixation in 1% OsO_4, and dehydration in ethanol. Coelomocytes for SEM were transferred into acetone and placed on glass cover slips which were coated with poly-L-lysine (Sanders *et al.*, 1975). The preparations were subjected to critical point drying (Denton DCP-1, Denton Vacuum Inc., Cherry Hill, NJ), coated with gold (PS-2 sputter coater, International Scientific Instruments, Glen Ellyn, IL), and viewed on a Stereoscan 600 SEM (Cambridge Scientific Instruments, Ossining, NY).

Cells used for TEM were transferred into propylene oxide, infiltrated, and embedded in plastic (Spurr, 1969). Sections were cut with a diamond knife on a Sorvall MT-1 ultratome and stained with uranyl acetate and lead citrate. Thin sections were viewed on an AEI-6B electron microscope operated at 60 kV.

Tiedemann's bodies were removed from animals which had been injected with 1 ml of the fixative solution. Excised organs were then prepared for TEM as described above.

Clearing of bacteria from coelomic fluid

Specimens of a marine, gram-negative short rod with a single polar flagellum, *Aeromonas* sp., UF-B (isolated and characterized by G. G. Holz, Jr., S.U.N.Y., Upstate Medical Center), were grown in an enriched sea water medium (Soldo and Merlin, 1972) at 37°C for 1 day. Bacteria were concentrated by centrifugation at 2500 × *g* and washed with M.B.L.-formula sea water. Approximately 3.5×10^7 bacteria in 1 ml of sea water were injected into the coelomic cavity of each sea star tested. Coelomic fluid was withdrawn at various time intervals, and a 0.1 ml portion (undiluted or diluted 1 : 100) was spread on agar plates prepared with the bacterial growth medium. Bacteria numbers were obtained by colony counts following incubation of agar plates at 37°C for 2 days. Another 2 ml portion was prepared for TEM in the glutaraldehyde fixative described above.

Results

Surface of coelomocytes

The phagocytic amoebocyte is the dominant cell type in the coelomic fluid of *Dermasterias imbricata*. Smaller mononuclear, nonflagellated cells have also been observed. The dynamic nature of the living coelomocytes was apparent by phase microscopy of the cells. Petaloid (bladder), lamellipodial, and filopodial forms were observed. The filopodial form was predominant when cells were allowed to adhere to glass slides. Coelomocytes were in petaloid or lamellipodial configurations when cells were observed immediately after coelomic fluid was sampled, or when cells were fixed immediately upon withdrawal of coelomic fluid from the sea stars. Surfaces of living coelomocytes rapidly and constantly changed shapes, forming pseudopodial extensions that quickly collapsed as still others formed.

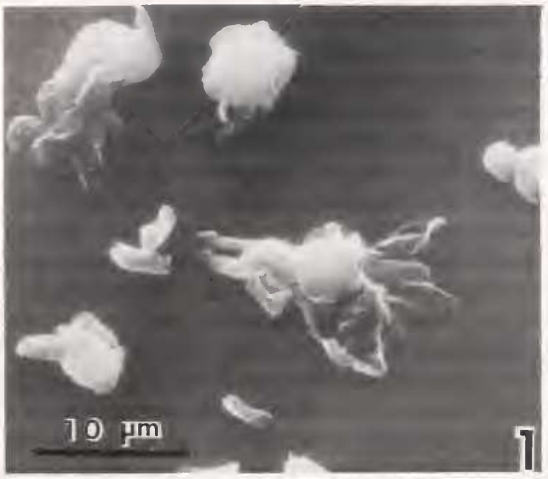
Cells prepared for SEM and TEM were fixed within seconds of withdrawal of coelomic fluid. Observations with SEM (Fig. 1) confirmed light microscopic studies which indicated that the lamellipodial or petaloid configurations were probably characteristic of these cells *in vivo*. Cells from one animal not only had extensive pseudopods, but also had microvilli protruding over the cell surfaces (Fig. 2).

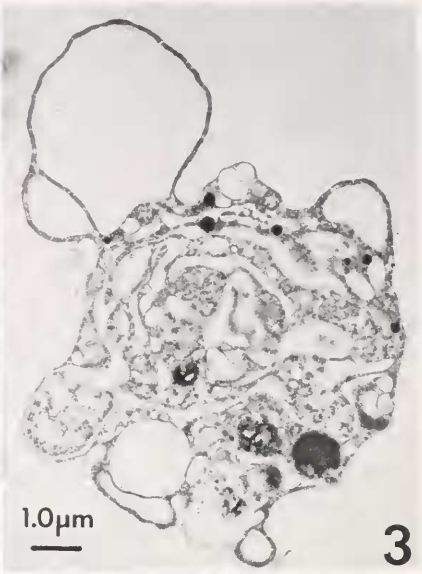
In thin sections viewed by TEM the surface membranes of these cells were greatly folded. Extensions that formed bladders were present in numerous cell profiles (Fig. 3). The extensive folding of the surface made it difficult to distinguish surface membranes from what appeared to be "clear" vesicles.

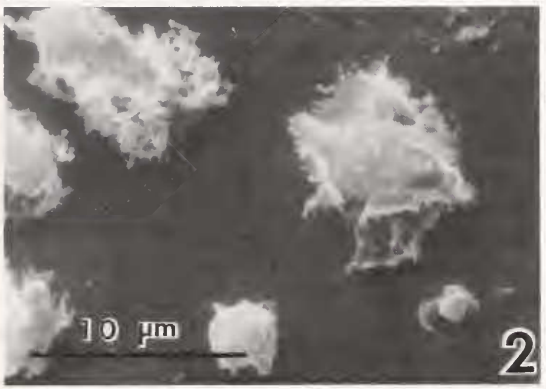
Lysosomes

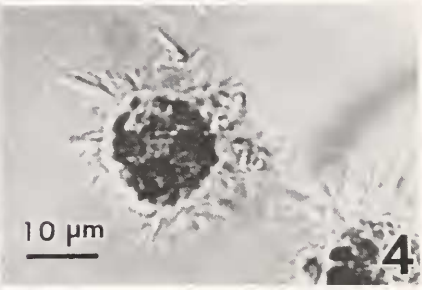
The cytoplasm contained many lysosomal vesicles. Cells stained with neutral red (Fig. 4) and viewed by light microscopy showed large numbers of granules, positive for the stain, concentrated in the central region of the cells. These granules were not observed in pseudopodial extensions. Cells in Figure 4 were photographed after they adhered to the microscope glass slide and hence displayed the filopodial configuration. In TEM the type of vesicles located centrally in coelomocytes, and seen in large numbers within a cell, contained electron-dense material surrounded by less dense material (Fig. 5). These vesicles were of various densities, perhaps reflecting their stage of development. The denser areas within these vesicles may be regions of condensation or "crystallization" of material packed within the vesicles (Fig. 6).

Another structure, which may be related to a stage in the maturation of these vesicles (or may be an artifact of sample preparation), is shown in Figure 7. These very dense structures often contained a clear area which in turn contained material of even greater density than the main structure. These very dense structures are probably also lysosomes, since such structures are associated with rod-shaped material (Fig. 8) that resembles the material within lysosomal vesicles (*cf.* Fig. 6). Hence, vesicles numerous in the cell and containing dense material surrounded by less dense matrix material are probably the lysosomal neutral-red granules observed by light microscope cytochemical methods. Furthermore, structures resembling the dense rod-shaped material of these vesicles occur in digestive vacuoles (see below) and support our interpretation of the identification and function of these organelles in the cell.









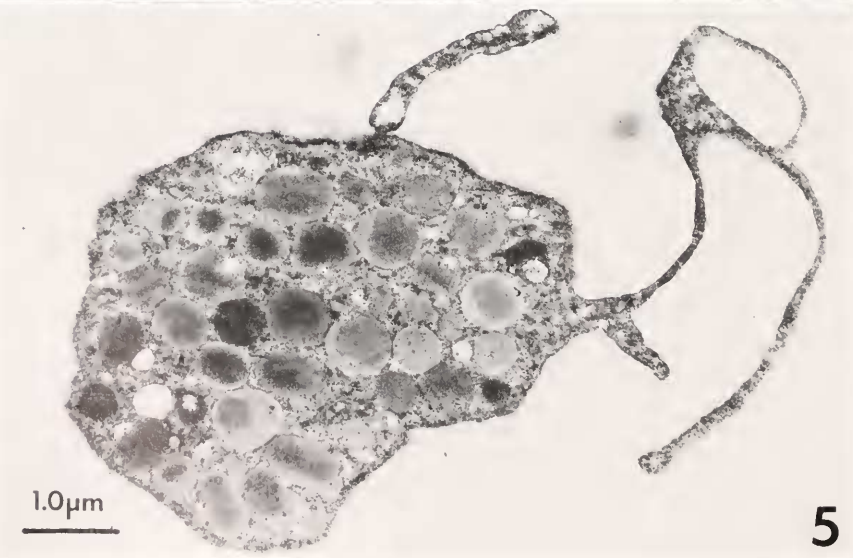


FIGURE 1. Scanning electron micrograph of coelomocytes of *Dermasterias imbricata* showing lamellipodial extensions of the cell surface. × 2000.

Other cell organelles

Where surface extensions formed lamellipods, the protrusions were filled with microfilaments (Fig. 9). Thick linear bundles of filaments in the cytoplasm radiated to the surface of the pseudopod. Organelles such as lysosomes and the nucleus were excluded from these protrusion zones.

The nucleus of coelomocytes was usually bean-shaped or notched, although various profiles were seen because of varying section planes (Fig. 9). Coelomocytes from normal and experimental sea stars were treated with Giemsa stain and observed by light microscopy at various times following removal of coelomic fluid. No nuclear division or mitotic activity was observed.

A Golgi complex was often seen in the notched region of the nucleus as well as in other parts of the cytoplasm. Golgi complexes were generally well developed and had several evenly and closely spaced stacks of lamellae (Fig. 10). Mitochondria were elongate (Fig. 11).

Coelomic fluid samples contained a few cells which had centrioles (basal bodies) associated with striated rootlets (kinetodesmal fibers) (Fig. 12). The combination of these structures suggested that those cells were ciliated or flagellated. These may have been cells normally at other sites but moved during coelomic fluid sampling by a hypodermic needle and syringe. On the other hand these cells may be analogs to the "vibratile" cells described as unique to sea urchins (Johnson, 1969b). *Strongylocentrotus* "vibratile" cells move by a single flagellum in the coelomic fluid. Also uncommon within coelomic fluid samples was a cell with a large vesicle filled with filamentous material (Figs. 13, 14). Again this may not be an *in situ* cell of the coelomic fluid. It is also possible that this cell normally can come from, or go to another tissue such as the Tiedemann's bodies (see below).

Phagocytosis

Washed suspensions of a pure culture of a marine *Aeromonas* were injected into sea star coeloms to characterize the phagocytic activity of coelomocytes. Coelomocytes were prepared for TEM at various periods from 5 min to 3 days after injection. By 5 min after the injection, coelomocyte morphology had changed. Large numbers of vacuoles contained structures undergoing digestion (Fig. 15). Bacteria engulfed by pseudopodial activity (Fig. 16) were observed within phagosomes in the cell (Fig. 17). Some bacteria in phagocytic vacuoles were lysed, indicating that digestion had begun (Fig. 18). Vacuoles containing heterogeneous structures were present (Fig. 19). The vacuoles in Figure 19 also contained dense material typical of the lysosomal matrix (see above). Myelin-like figures, indicative of cellular digestive processes, were abundant. These myelin-like figures were present in vesicles (Fig. 20), large vacuoles (Fig. 21),

FIGURE 2. *Dermasterias imbricata* coelomocytes with microvilli protruding from their surfaces. Most cells in these samples had microvilli. × 2780.

FIGURE 3. Thin section of a coelomocyte, showing the bladder or petaloid surface morphology. Note the highly folded surface membrane and numerous cytoplasmic vesicles. × 6750.

FIGURE 4. Light micrograph of a coelomocyte stained for lysosomes with neutral red. The cell had adhered to the microscope glass slide and thus exhibits filopodial surface extensions. Neutral red-stained granules are restricted to central regions of the cell and do not extend into filopods. × 920.

FIGURE 5. Section through a coelomocyte showing high concentrations of lysosomal vesicles. × 13,390.

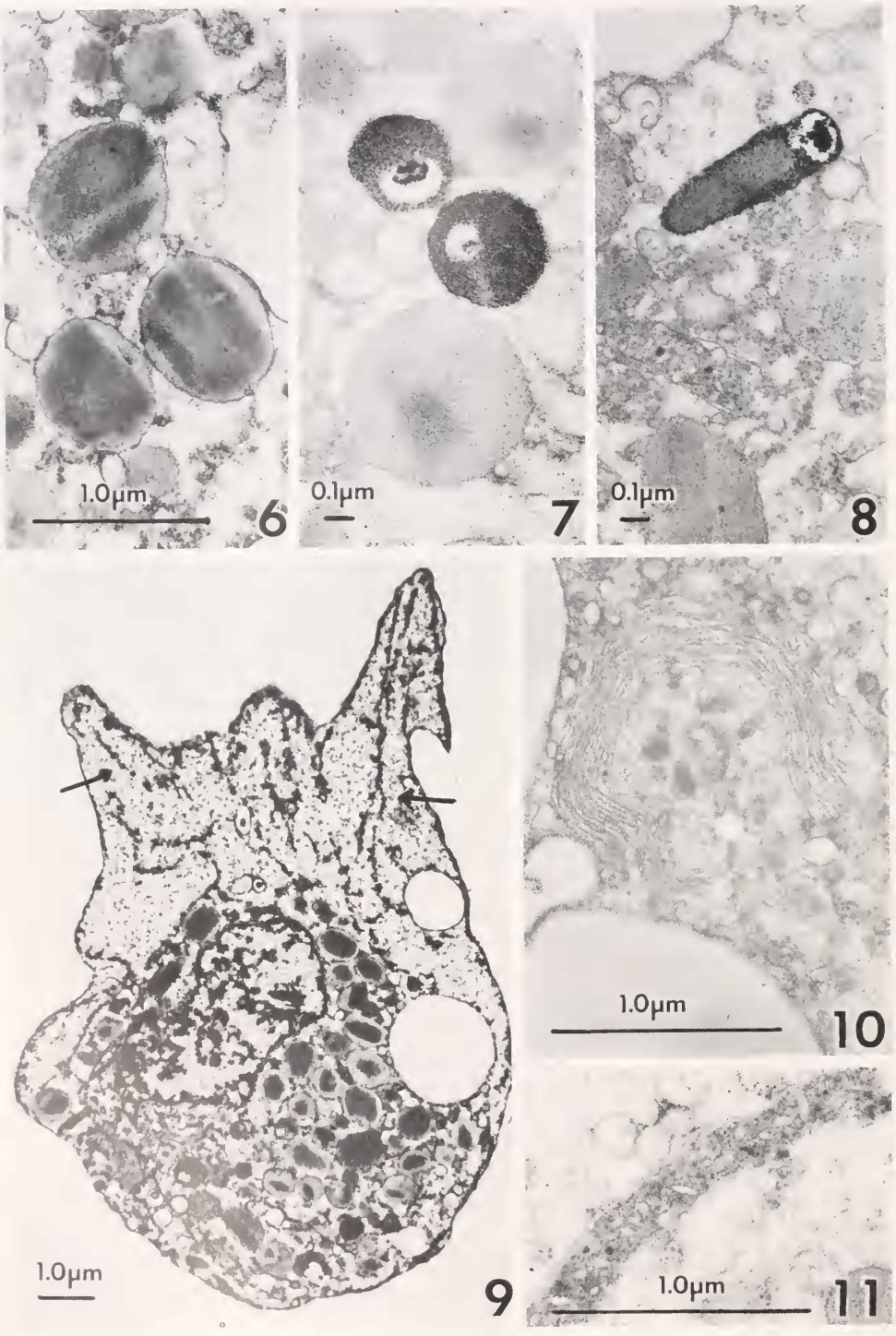


FIGURE 6. Lysosomal vesicles containing more condensed or electron-dense material surrounded by less dense matrices. Denser material often appears in rod-like profiles. This cell was taken from an animal 60 min after bacteria were injected into the coelom. Lysosomal vesicles are similar in cells of untreated animals. × 24,700.

FIGURE 7. Dense vesicles containing clear regions in the cytoplasm. The clear regions contain very dense material (*cf.* more typical lysosomal vesicles next to them and in Fig. 6).

and cell cytoplasm. In many cell profiles it was not possible to determine whether structures that appeared to be related to digestion were distinct phagosomes (secondary lysosomes) or autophagous structures. We did not determine whether coelomocytes self-destruct after they perform their phagocytic function, recover and remain in the coelomic fluid, or are removed from the coelomic fluid.

Aeromonas introduced into the coelomic fluid of sea stars by direct injection of a bacterial suspension were effectively cleared from the coelomic fluid within 2–3 days. No bacterial colonies formed when 0.1 ml of coelomic fluid, withdrawn before injection of bacteria, was plated. This indicates that the coelomic fluid is normally aseptic. Figure 22 shows the rate of disappearance of bacteria from the coelomic fluid of two animals. Also shown is the concentration of coelomocytes in these animals over this time period.

Relationship with the Tiedemann's bodies

Tiedemann's bodies may serve as sites from which coelomocytes arise, through which coelomocytes pass, or where coelomocytes can associate. Our studies do not establish coelomocyte genesis but do suggest a possible relationship and some similarities between cells of the organ and circulating coelomocytes. Tiedemann's bodies are located on the inner side of the peristomial ring and are outpockets of the ring canal of the water vascular system. The paired bodies are approximately 2 mm in diameter and are hollow and highly folded. The folds are lined with columnar or squamous epithelial cells (Hyman, 1955). Figure 23 is a section through an infold showing the lining of epithelial cells and a cell (coelomocyte) in the lumen. The animal was not injected with bacteria but clearly contained numerous bacteria that were probably present in the natural environment and/or the laboratory aquarium. Bacteria were identified in phagocytic vacuoles (Fig. 23) and also within vesicles that resembled lysosomes, *i.e.* appeared to be filled with matrix material (Fig. 24). Some profiles of epithelial cells show notched nuclei (Fig. 23) suggesting that these cells have the same nuclear configuration as do nuclei of coelomocytes. A cell (presumably a coelomocyte) within the lumen, which is contiguous with the water vascular system, contained a large vacuole with a structure having filamentous material (Figs. 25, 26) identical to that observed in a cell in the coelomic fluid (see above and Fig. 13). The nature of the filamentous material is not known, but it resembles smooth muscles seen in Tiedemann's bodies. These observations suggest that coelomocytes can migrate from coelomic fluid, which bathes the exterior of Tiedemann's bodies, to the interior of the organ, which is part of the water vascular system.

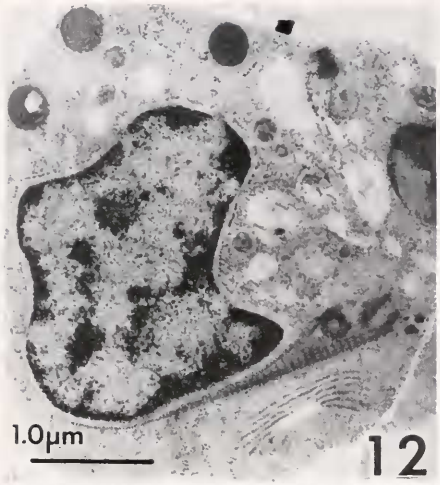
This cell was taken from an animal 5 min after bacteria were injected into the coelomic cavity. These structures are also present in cells of untreated animals. × 39,110.

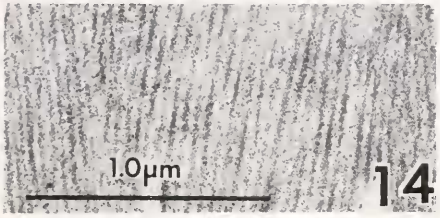
FIGURE 8. A vesicle with structures resembling those shown in Figures 6 and 7. The rod-shaped element within the very dense matrix suggests these are lysosomes in different stages of maturity. This cell was taken from an animal 5 min after bacteria were injected into the coelom. × 37,660.

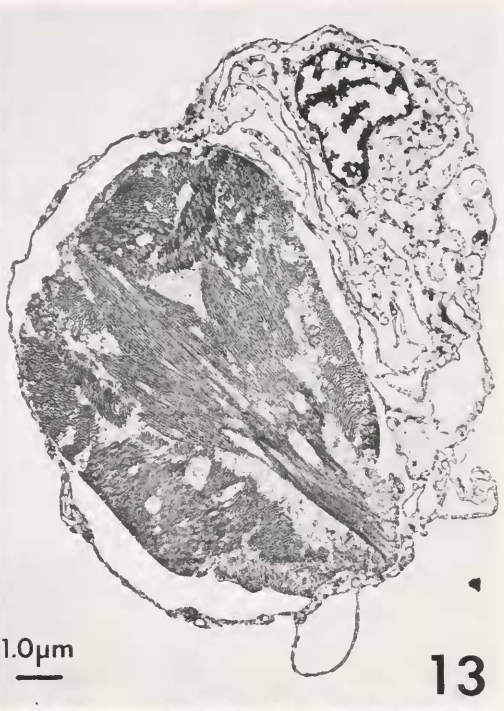
FIGURE 9. A cell with filamentous material in pseudopodial protrusions. Thick bundles (arrows) extend from the central region of the cell to the cell surface. Organelles such as lysosomes and the nucleus are excluded from protrusion zones. This cell was taken from an animal 5 min after bacteria were injected into the coelomic fluid. × 8410.

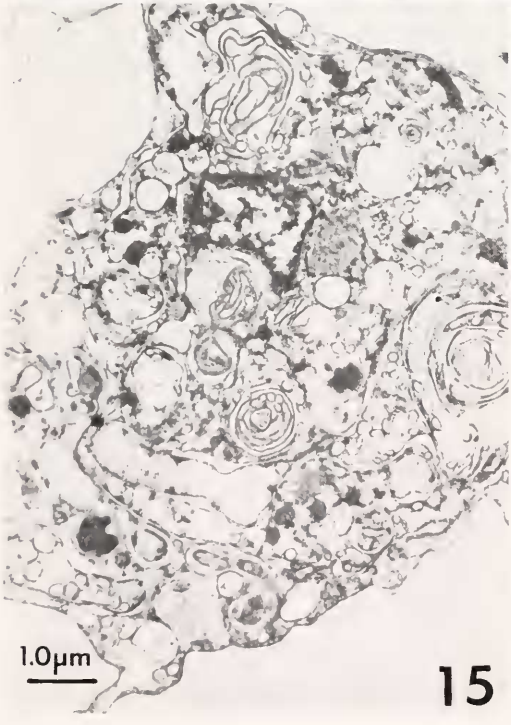
FIGURE 10. A Golgi apparatus in the cytoplasm of a coelomocyte. Lamellae are even and numerous vesicles are present on the convex mature face of the complex. This cell was taken from an animal 5 min after it was injected with bacteria. Golgi complexes are the same in cells of untreated animals. × 32,880.

FIGURE 11. A mitochondrion sectioned along its length. The outer membrane and cristae are visible. Electron-dense deposits (also see mitochondria in Fig. 8), probably divalent cation deposits, are present. This cell was taken from an animal 60 min after it was injected with bacteria. Mitochondria have the same morphology in cells of untreated animals. × 37,180.









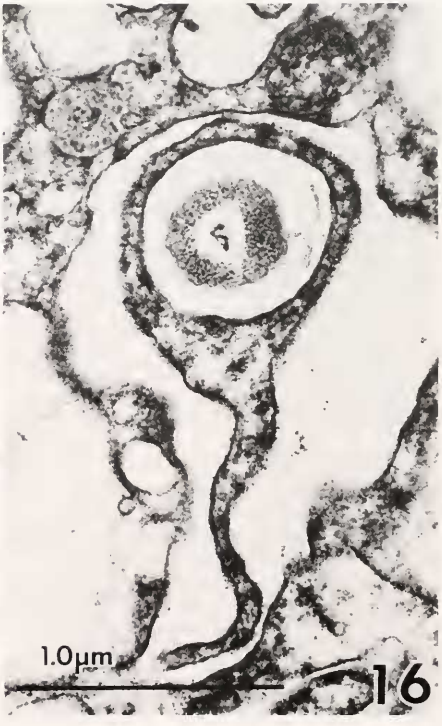


FIGURE 12. A cell with basal bodies and striated rootlets, from a coelomic fluid sample. The rootlet appears to associate with the nucleus. ×16,320.

FIGURE 13. A cell with a large vacuole filled with filamentous material from a coelomic fluid sample (*cf.* Figs. 25, 26). The nature of the filamentous material is not known. This cell was taken from a sea star 60 min after the animal was injected with a bacterial suspension. × 6300.

Some cells within the Tiedemann's bodies were flagellated (Fig. 27) and these organelles were associated with elaborate striated rootlets. As described above, we have observed a cell in the coelomic fluid with a basal body and rootlet.

Concentration of coelomocytes in coelomic fluid

Concentration of coelomocytes varied widely from animal to animal. Cell counts ranged from 1.5×10^5/ml to 1.2×10^7/ml in 57 untreated sea stars. There was no obvious correlation of cell number with the animal's size. Similar heterogeneity probably occurs in the natural population. A wide range of percent change in coelomocyte concentration was observed after withdrawal of 7.5 ml (experimental) or 0.5 ml (control) coelomic fluid. Increased concentrations of coelomocytes were observed in 3 out of 6 animals after 1 day; 9 out of 11 after 2 days; 12 out of 15 after 3 days; and 5 out of 10 after 4 days. Some animals did not show any change and a few showed lower coelomocyte concentrations.

Weights of animals taken before withdrawal of 7.5 ml fluid and weights taken at 3 or 4 days after coelomic fluid removal indicated that the animals regained or increased their total body weights. At day 3 there was a mean increase of 5.3 g ($\pm$ 8.6 SD, N = 18) and at day 4 a mean increase of 29.5 g ($\pm$ 11.5 SD, N = 8).

Discussion

Cell surface

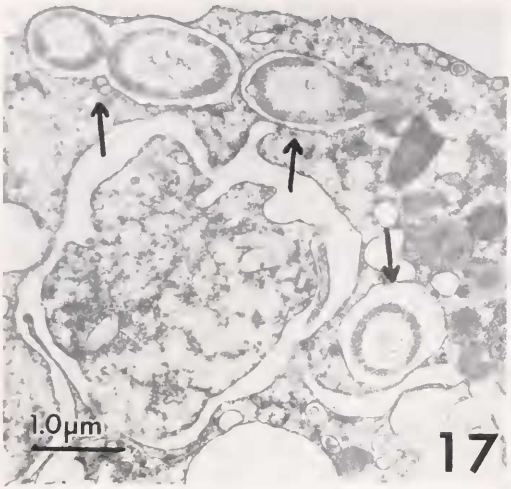
The coelomocytes of the sea star, *Dermasterias imbricata,* have the same general morphology as those reported in echinoids and holothurians (Johnson, 1969a, b, c; Fontaine and Lambert, 1973, 1977). Transformation from lamellipodial or petaloid forms to the filopodial form was observed when cells adhered to glass slides. This transformation has recently been carefully documented in echinoid coelomocytes by Edds (1977) who detected reorganization of microfilament bundles when cells were allowed to settle on glass. The cell shape changes are probably controlled by an actin-myosin-based system, since microfilaments are numerous in pseudopods. Actin has been isolated from echinoid coelomocytes in milligram quantities and its migration in gels characterized (Edds, 1977, 1979; Otto *et al.,* 1979). Echinoid coelomocytes change from petaloid to the lamellipodial forms upon settling on a glass substratum and then to the filopodial form upon hypotonic shock (Otto *et al.,* 1979). The transformation of *Dermasterias* coelomocytes to the filopodial form apparently did not require hypotonic shock, since the change occurred in cells in undiluted coelomic fluid.

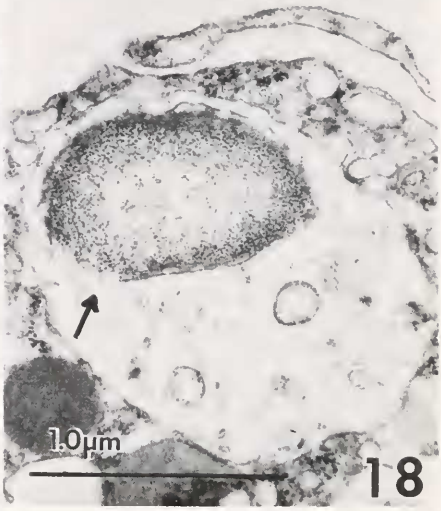
Microvilli of various cell types are also known to contain microfilaments and actin-like molecules (Mooseker and Tilney, 1975). Our observations that "villous" structures can appear on surfaces of sea star coelomocytes indicate that these cells have the same capacities as vertebrate macrophages and lymphocytes to produce microvilli (Cohn, 1968). Whether or not smooth *vs.* "villous" surface morphologies were the results of fixation procedures was not examined in this study. The usual "villous" surface morphology of human T- and B-lymphocytes appear

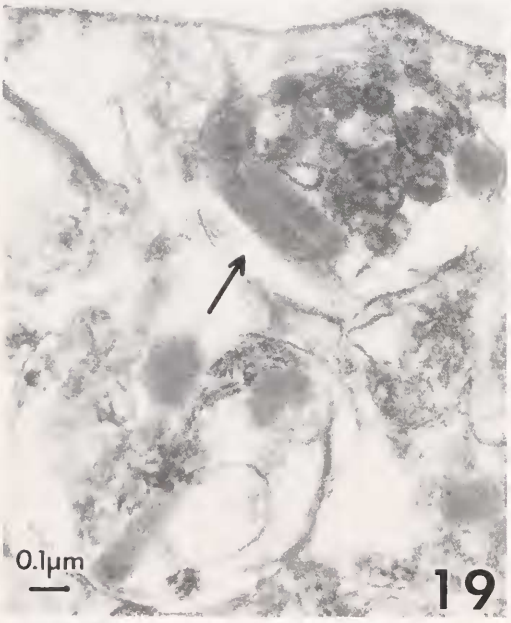
Figure 14. A higher magnification of the filamentous material shown in Figure 13. × 31,790.

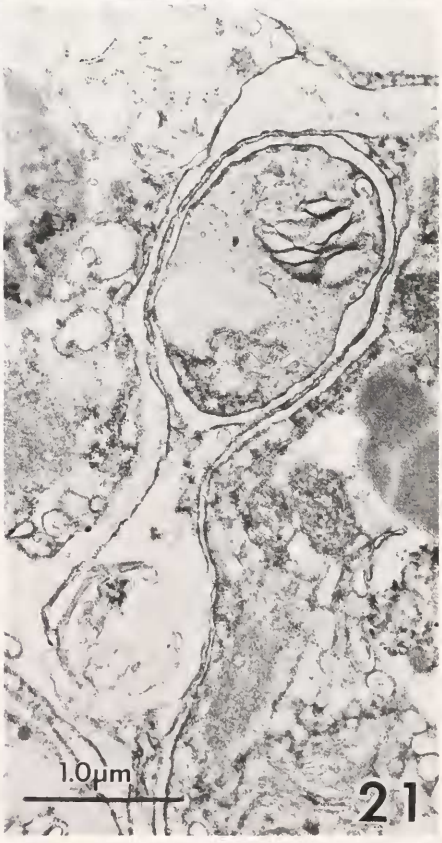
Figure 15. A coelomocyte 60 min after bacteria were injected into the coelomic cavity of the sea water. Bacteria are in vacuoles and digestive processes are indicated by the presence of myelin-like figures, and by numerous vacuoles of mixed contents. ×9100.

Figure 16. A bacterium being "lassoed" by pseudopodial activity of the coelomocyte surface. This sample of coelomic fluid was taken 5 min after bacteria were introduced into the coelom. × 35,200.









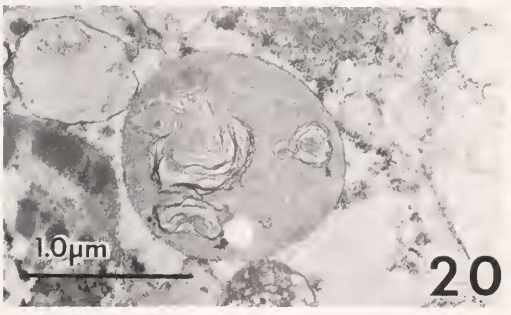


FIGURE 17. Several bacteria (arrows) within phagocytic vesicles 5 min after bacteria were introduced into the coelomic cavity of sea stars. × 12,530.

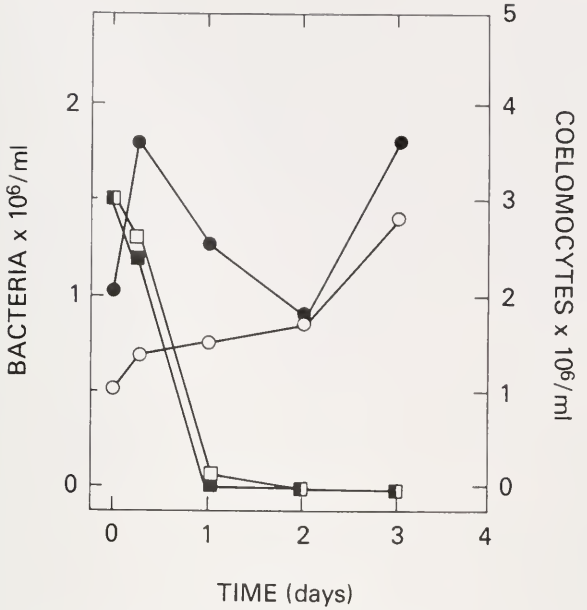


FIGURE 22. The rate of clearing of bacteria from the coelomic fluid of two sea stars (open and closed squares) and concentration of coelomocytes in the two animals (open and closed circles). Animals with bacteria-free coelomic fluid were injected with 3.5×10^7 cells of *Aeromonas* sp.

smooth after various fixation procedures (Alexander *et al.*, 1976). Our observations establish that unlike many cell types, these coelomocytes can exhibit microvilli-covered surfaces when prepared for and viewed by SEM.

Phagocytosis

Numerous studies indicate that echinoderm coelomocytes can actively phagocytize and clear foreign material within a few days after its injection (Bang and Lemma, 1962; Endean, 1966; Johnson, 1969c; Johnson *et al.*, 1970; Reinisch and Bang, 1971; Unkles and Wardlaw, 1976; Wardlaw and Unkles, 1978; Coffaro, 1978). *Dermasterias*, whose normal coelomic fluid was found to be aseptic, also rapidly disposed of injected gram-negative bacteria. Johnson *et al.* (1970) provided ultrastructural evidence that coelomocytes from *Strongylocentrotus* phagocytize bacteria *in vitro* in hanging drops. The cells, however, did not have recognizable primary lysosomes and bacterial cells taken up did not appear

FIGURE 18. A bacterium within a phagosome shows signs of being digested. The arrow points to a broken region of the bacterial surface. This cell was taken from the animal 60 min after the animal was injected with bacteria. × 33,860.

FIGURE 19. Large digestive vacuoles are common in the cytoplasm of cells taken 60 min after sea stars were injected with bacteria. The vacuoles contain rod-shaped elements (arrow) similar in density and character to those seen in lysosomes (*cf.* Fig. 6). × 53,210.

FIGURE 20. Vesicles with homogeneous matrix material similar to that seen in lysosomes contained myelin-like figures. This vesicle was in a cell taken 5 min after the animal was injected with bacteria. × 22,160.

FIGURE 21. Vacuoles containing myelin-like figures in a cell taken 5 min after the animal was injected with bacteria. × 21,030.

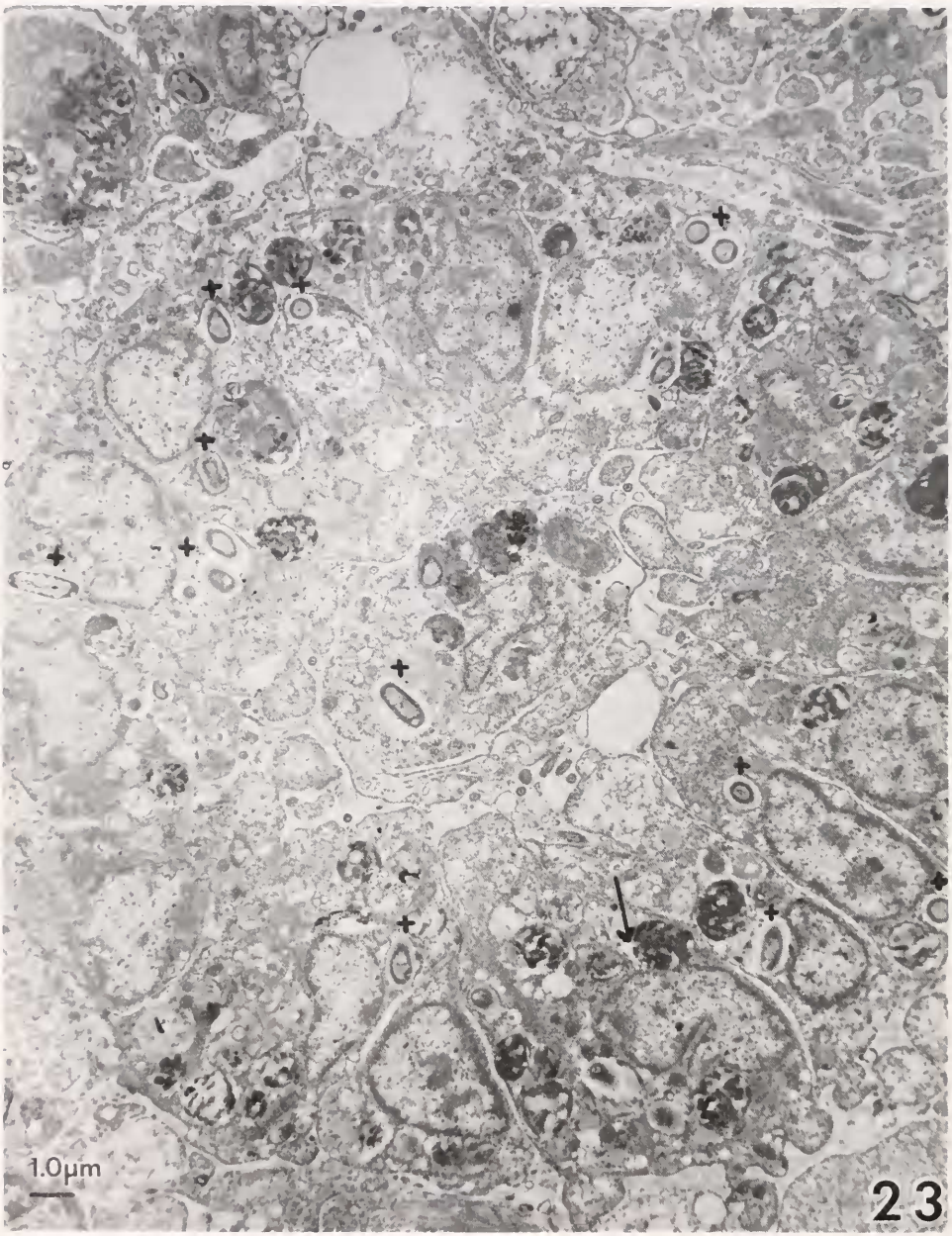
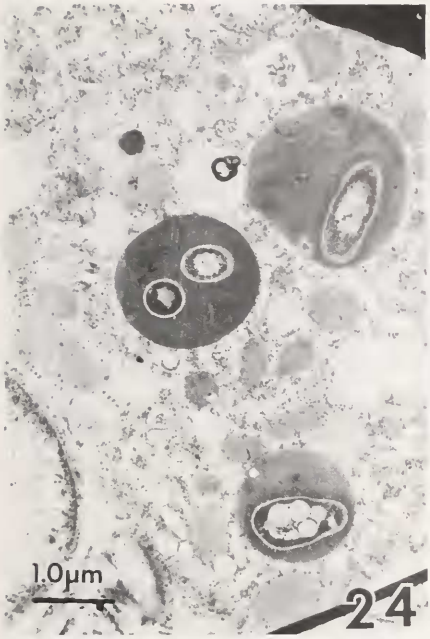
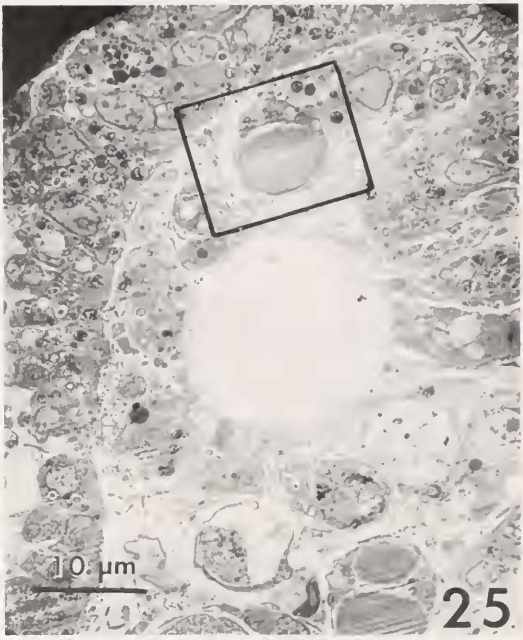
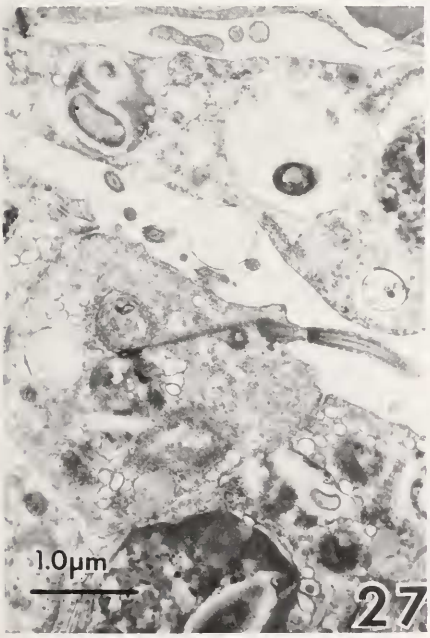


FIGURE 23. A section through a fold within a Tiedemann's body removed from an untreated sea star. Epithelial cells form a circle around a cell in the lumen. The arrow points to an epithelial cell sectioned at a notched region of the nucleus. Crosses indicate bacteria within vesicles in epithelial cells, and within the cell (presumably a coelomocyte) in the lumen. × 6380.

degraded. Several other studies (Bang and Lemma, 1962; Ghiradella, 1965; Endean, 1966; Reinisch and Bang, 1971) have reported that coelomocytes phagocytize foreign matter and then clump within dermal papulae (branchiae). These







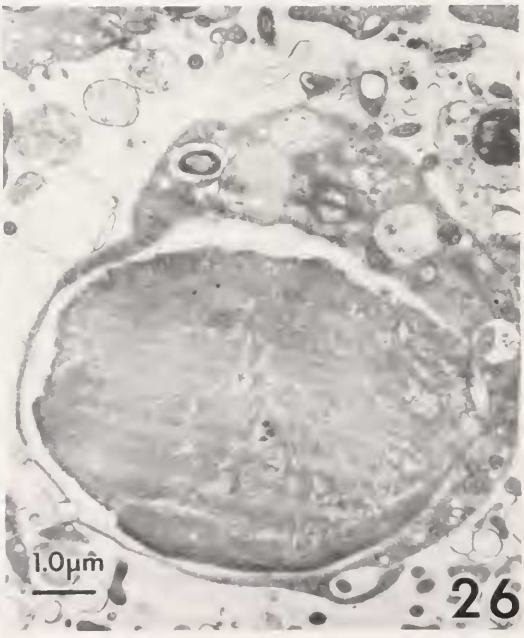


FIGURE 24. Lysosome-like vesicles within a Tiedemann's-body cell. These vesicles with homogeneous matrices contain bacteria. × 10,900.

FIGURE 25. Low magnification of a section through a fold in a Tiedemann's body. The rectangle indicates a cell in the lumen. × 1570.

FIGURE 26. A higher magnification of the cell in the lumen (marked in Fig. 24). This cell has a large vacuole filled with filamentous material (*cf.* Fig. 13). × 8340.

FIGURE 27. A flagellated cell in the Tiedemann's body. The pair of basal bodies and associated striated rootlet are similar to that seen in a cell in a sample of coelomic fluid. × 14,370.

structures then rupture, releasing the coelomocytes to the exterior. Our ultrastructural observations suggest that coelomocytes undergoing extensive phagocytic activity not only are filled with digestive vacuoles but may also participate in autophagous activity. Since the initial bacterial concentration was less than the coelomocyte concentraton, not all digestive vacuoles may have been involved with bacterial elimination. Autophagy in these cells suggests that spent phagocytic coelomocytes of *Dermasterias* self-destruct in the coelomic fluid. Another possibility is that they are expelled from the animal, as observed in other echinoderms (Bang and Lemma, 1962; Endean, 1966; Reinisch and Bang, 1971). It is interesting that the coelomocytes described here show a great deal of similarity to vertebrate macrophages in that they: 1) can produce microvilli on their surfaces, 2) have numerous lysosomes, well developed Golgi complexes (usually located in the nuclear "hof"), notched nuclei, and elongated mitochondria and 3) phagocytize foreign particles forming heterophagic as well as apparent autophagic vacuoles (*cf.* Cohn, 1968).

Tiedemann's bodies

Tiedemann's bodies are organs that may function as primitive lymphoid tissue in sea stars. The organs are outpockets of the water vascular system and are bathed on their outer surfaces by coelomic fluid. Hence, all major fluid systems of the organism circulate around the cells of this organ. The cells of Tiedemann's bodies are capable of endocytosing bacteria. The lumina of the deep folds on the water vascular system side of the organ are filled with cells that resemble coelomocytes (*cf.* Hyman, 1955). The epithelial cells of the Tiedemann's bodies are flagellated, as are those of the radial and ring canals (Hyman, 1955), and possibly serve to move fluid in the water vascular system. Vanden Bossche and Jangoux (1976) have reported that asteroid coelomocytes originate from the coelomic epithelium including the epithelium of Tiedemann's bodies. Since dividing cells were not observed in the coelomic fluid, it appears that circulating coelomocytes of *Dermasterias* might also come from other tissue sources. Animals recovering from extensive withdrawal of coelomic fluid regained total body weights. If that recovery (or increase) in weight reflects coelomic fluid replacement, then the recovery (or increase) in coelomocyte concentrations must result from coelomocyte recruitment. It is possible that coelomocytes originate from epithelial cells, *e.g.* of Tiedemann's bodies. Vanden Bossche and Jangoux (1976) reported that epithelial cells of sea stars became detached and rapidly lost their flagella. The rare cells in coelomic fluid with basal bodies and striated rootlets may represent incompletely transformed recent recruits. The findings reported here reveal indirect evidence that this organ may contribute to the coelomocyte population as well as play an important role in the animal's defense system.

Phagocytic cells participate in rejection of integumentary allografts in *Dermasterias*. The rejection site is heavily infiltrated by large phagocytic cells and smaller mononuclear cells. Autografts do not contain these cell types (Karp and Hildemann, 1976). A number of recent findings implicate the phagocytic amoebocyte as the effector cell. Karp and Johns (1978) reported that coelomic amoebocytes can be stimulated *in vitro* by such mitogenic substances as bacterial lipopolysaccharide and concanavalin A. Bertheussen (1979) reported that the echinoid coelomic phagocyte was able to recognize and react to allogeneic and xenogeneic cells in culture. Other echinoid cell types did not show this activity. Various studies have reported that filopodial coelomocytes participate in "clot" formation

and in the elimination of foreign material (Karp and Coffaro, 1980). In addition, preliminary studies indicate that the coelomocyte of *Dermasterias* has surface proteins with molecular weights similar to vertebrate (human and murine) transplantation antigens (L. A. Rheins, J. D. Stinnett, and R. D. Karp, University of Cincinnati, unpublished results). Thus, the phagocytic coelomocyte may mediate specific immune defense reactions in the sea star, and possibly in echinoderms in general. This cell may provide insight into how the sophisticated immune mechanisms of vertebrates evolved.

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Summary

1. The structure of coelomocytes from *Dermasterias imbricata* was characterized by light microscopy and scanning and transmission electron microscopy. The petaloid or lamellipodial configurations were the prevalent forms in freshly drawn coelomic fluid. Cells produced filopodia when they adhered to glass slides.

2. Lysosomes were stained with neutral red and observed by light microscopy, and variations in density and structures of their matrices described by TEM.

3. Animals injected with a bacterial suspension cleared the bacteria from their coelomic fluid within 2–3 days. Ultrastructure of coelomocytes in these animals indicated that phagocytosis and digestion of bacteria were rapid and involved lysosomes.

4. With TEM numerous bacteria were observed within cells of Tiedemann's bodies from untreated animals, indicating that this organ may play an important role in clearing the coelomic fluid and water vascular system of foreign particles.

5. Giemsa-stained coelomocytes of untreated animals and animals recovering from coelomic fluid removal indicated that coelomocytes did not undergo mitosis within the coelomic fluid. Removal of coelomic fluid resulted in recovery or increase in total body weights and coelomocyte concentrations. Hence, circulating coelomocytes must be recruited from other tissue sources in the animal.

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